

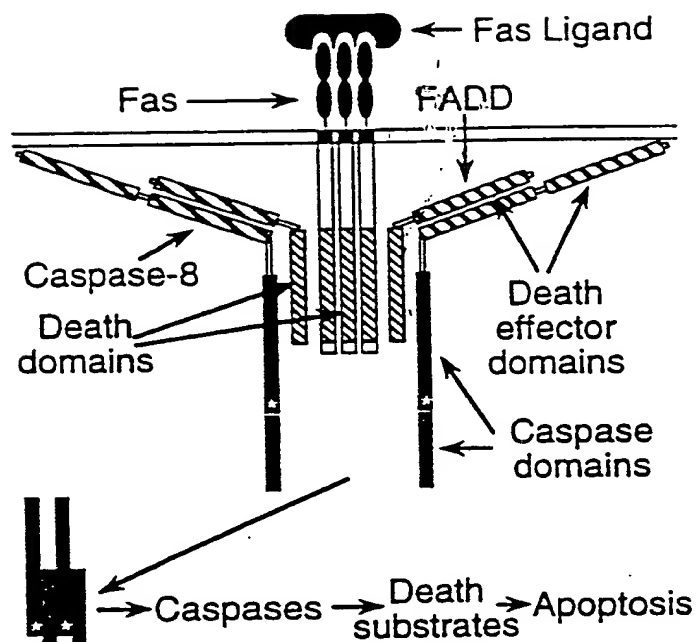
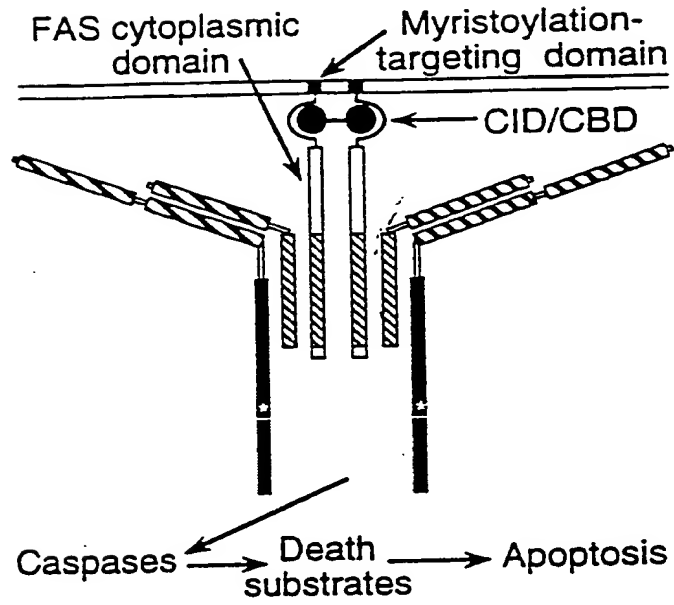
FIGURE 1**BEST AVAILABLE COPY**

FIGURE 2

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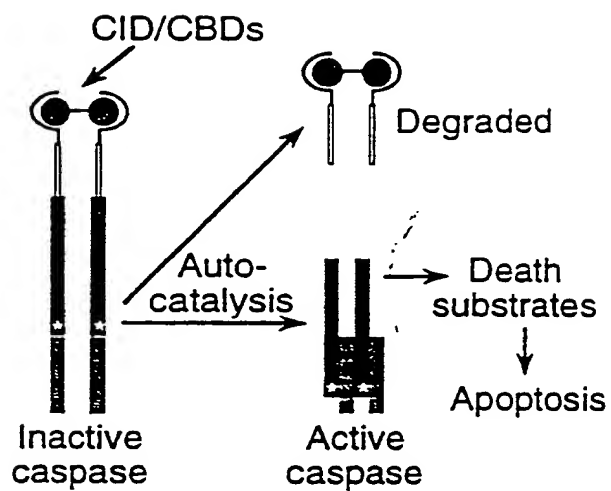
FIGURE 3

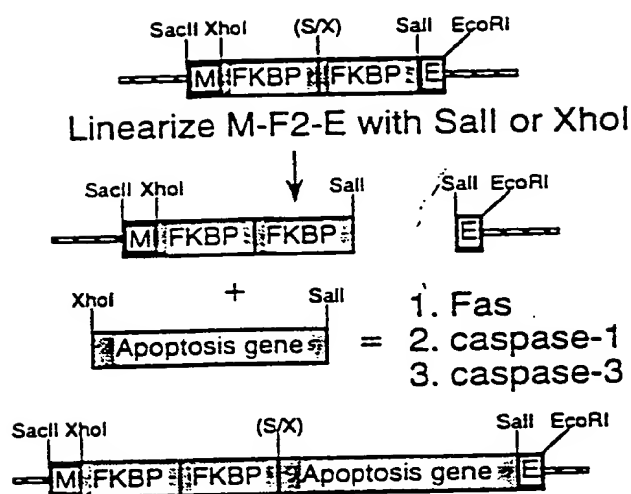
FIGURE 4

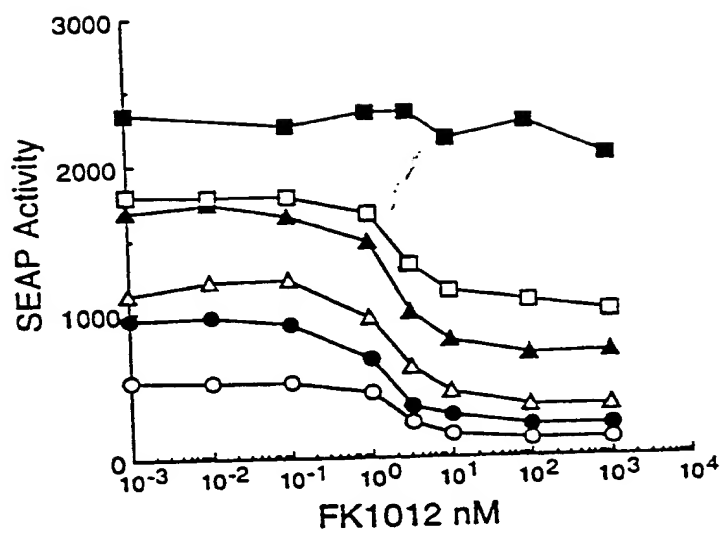
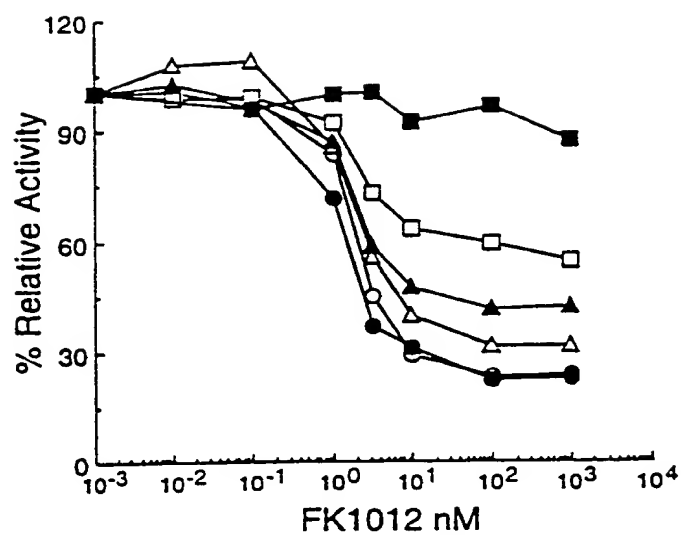
FIGURE 5

FIGURE 6

59/647418

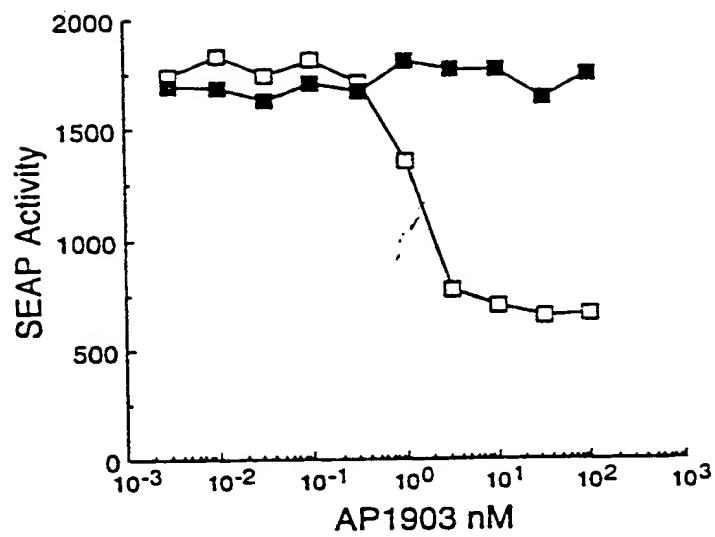
FIGURE 7

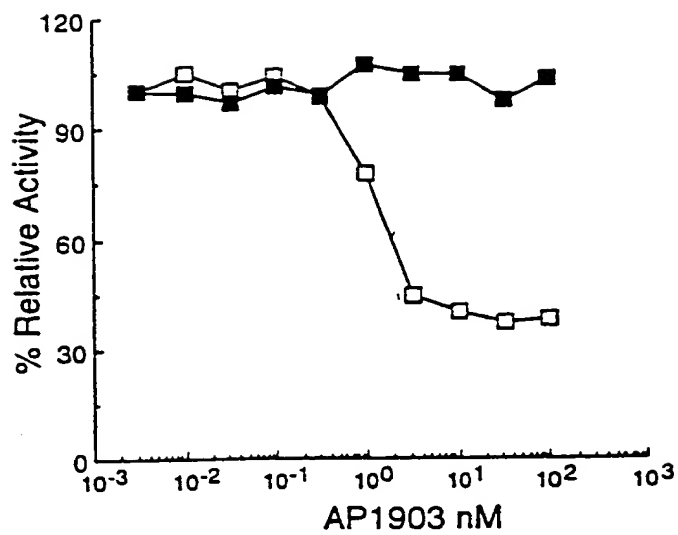
FIGURE 8

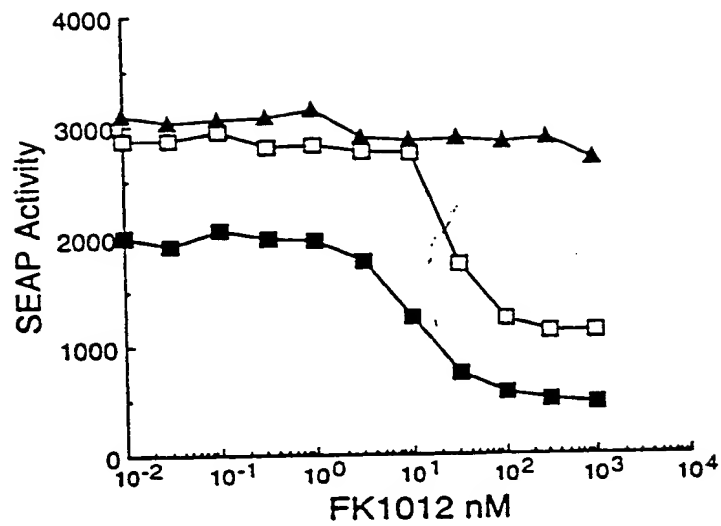
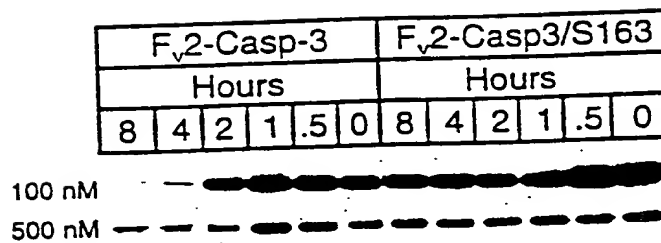
FIGURE 9

FIGURE 10

006260" 8T47960

FIGURE 11

F _v 2-Casp-3						F _v 2-Casp3/S163					
AP1903 nM						AP1903 nM					
100	32	10	3.2	1	0	100	32	10	3.2	1	0

09/647418-092900

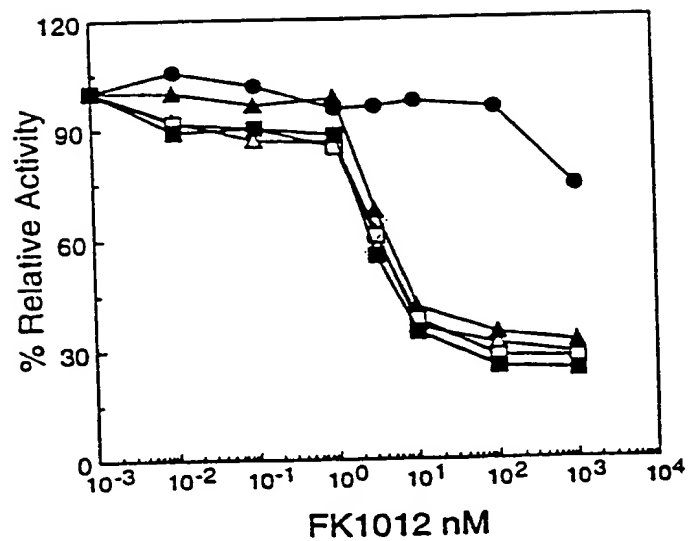
FIGURE 12

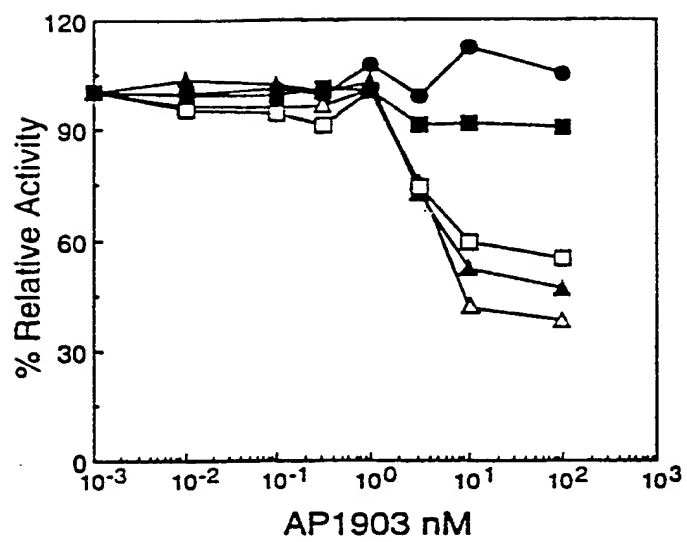
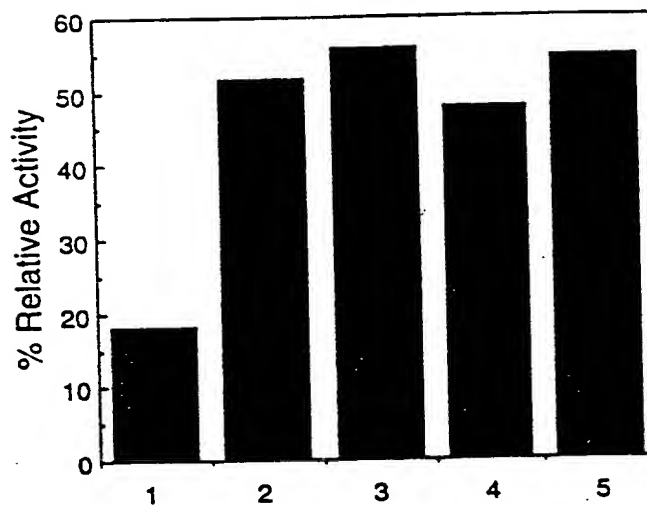
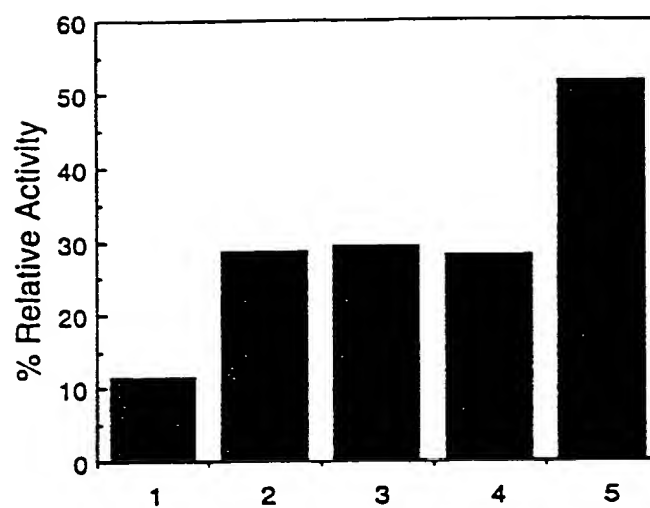
FIGURE 13

FIGURE 14

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FIGURE 15

006260" 8744950

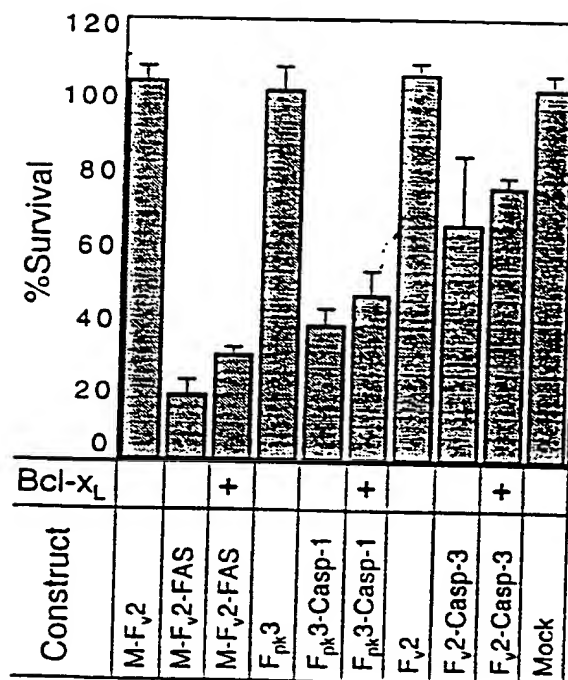
FIGURE 16

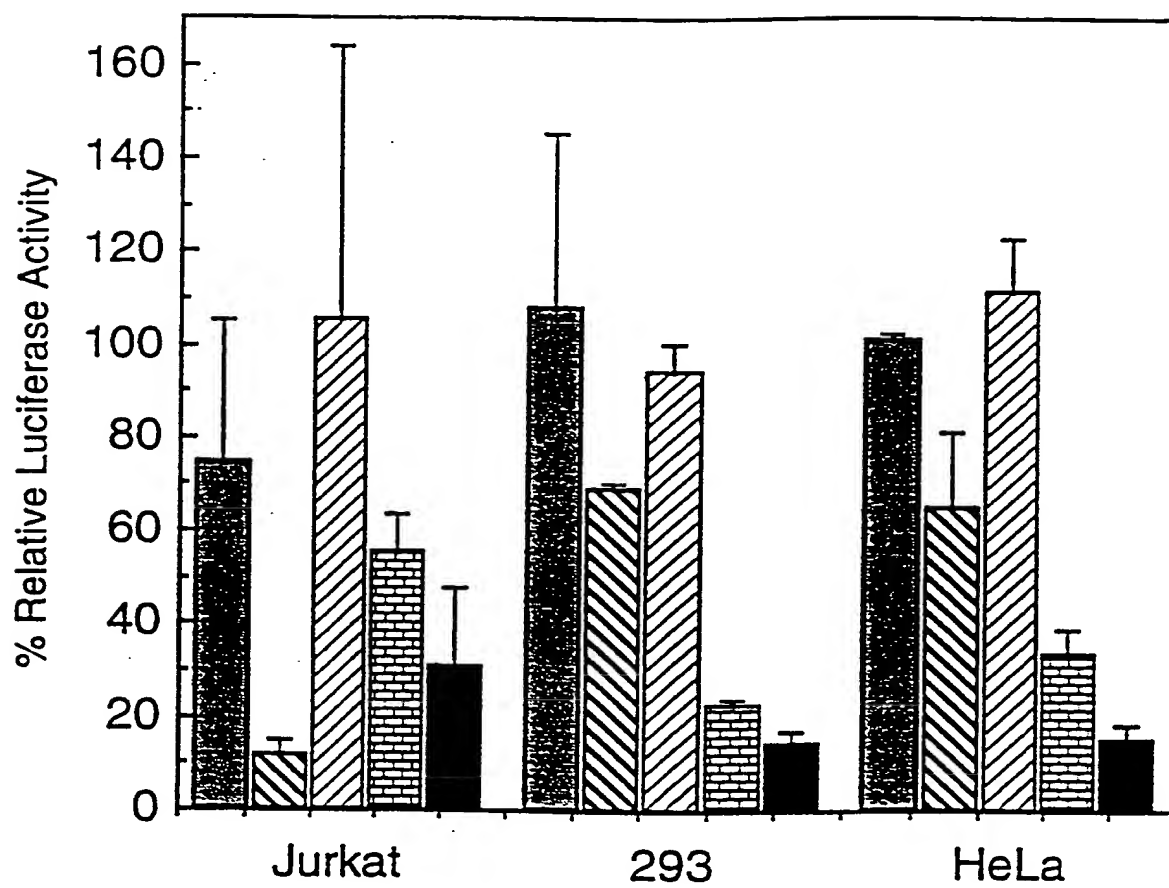
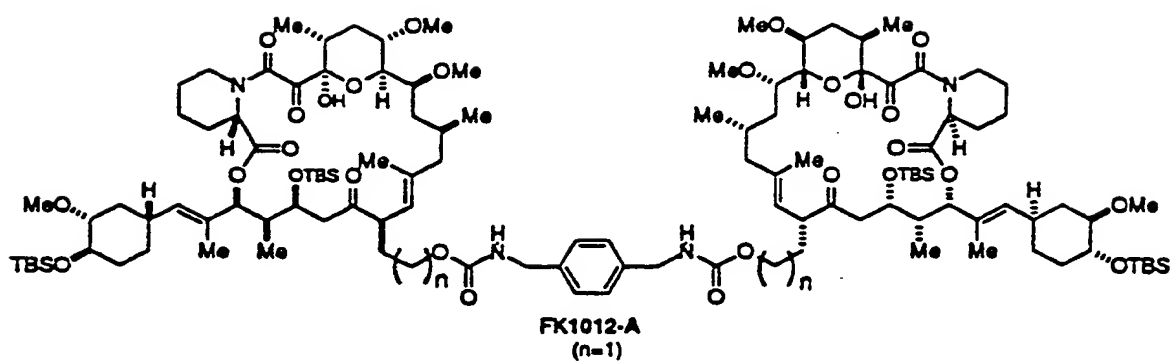
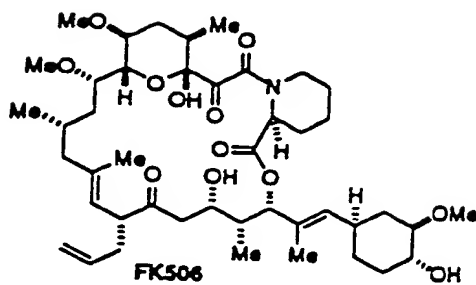
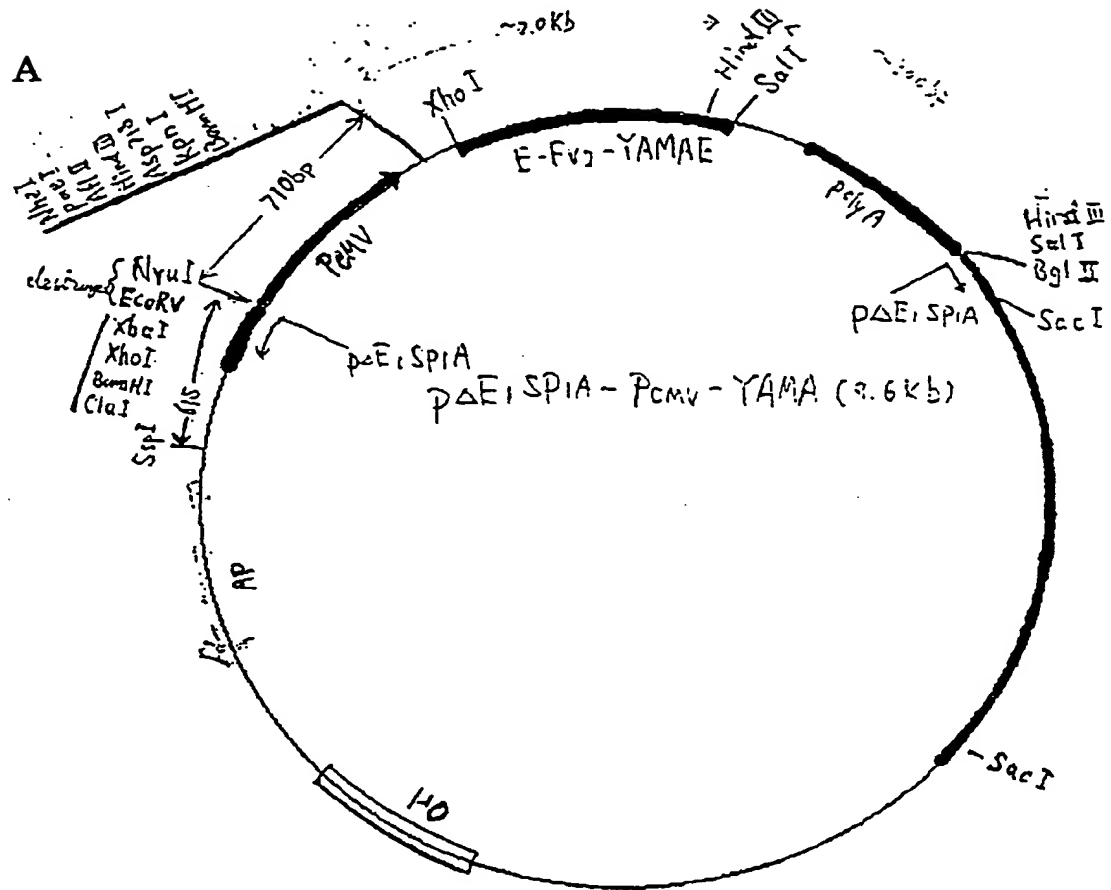
FIGURE 17

FIGURE 18



006260" 87424950

FIGURE 19

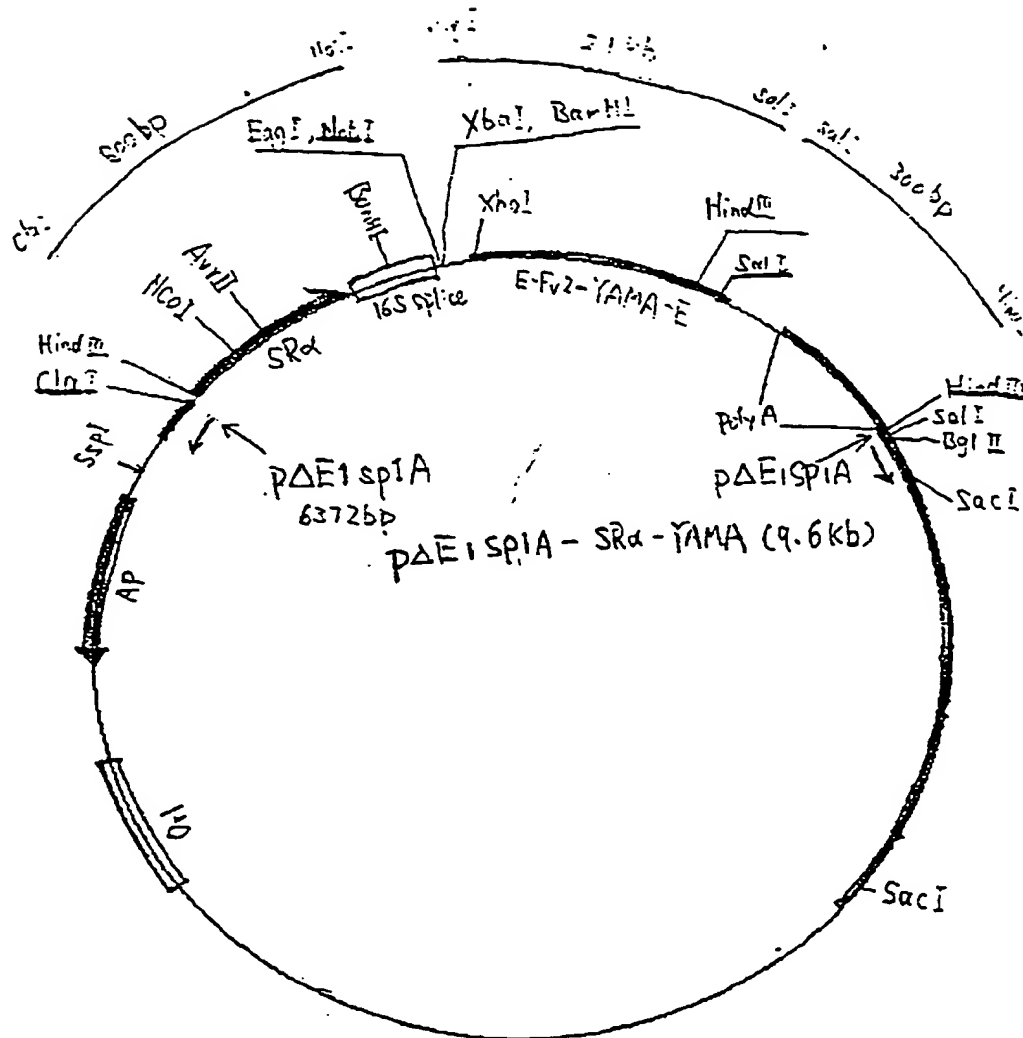


B



FIGURE 20

A



B

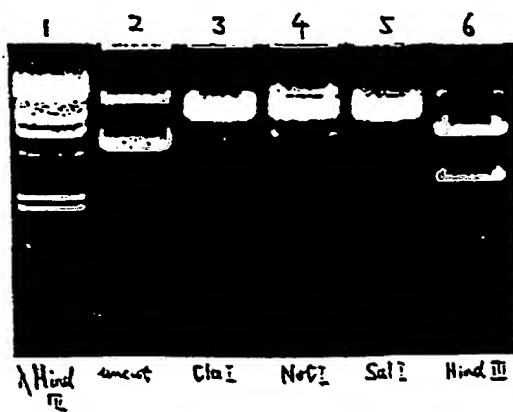
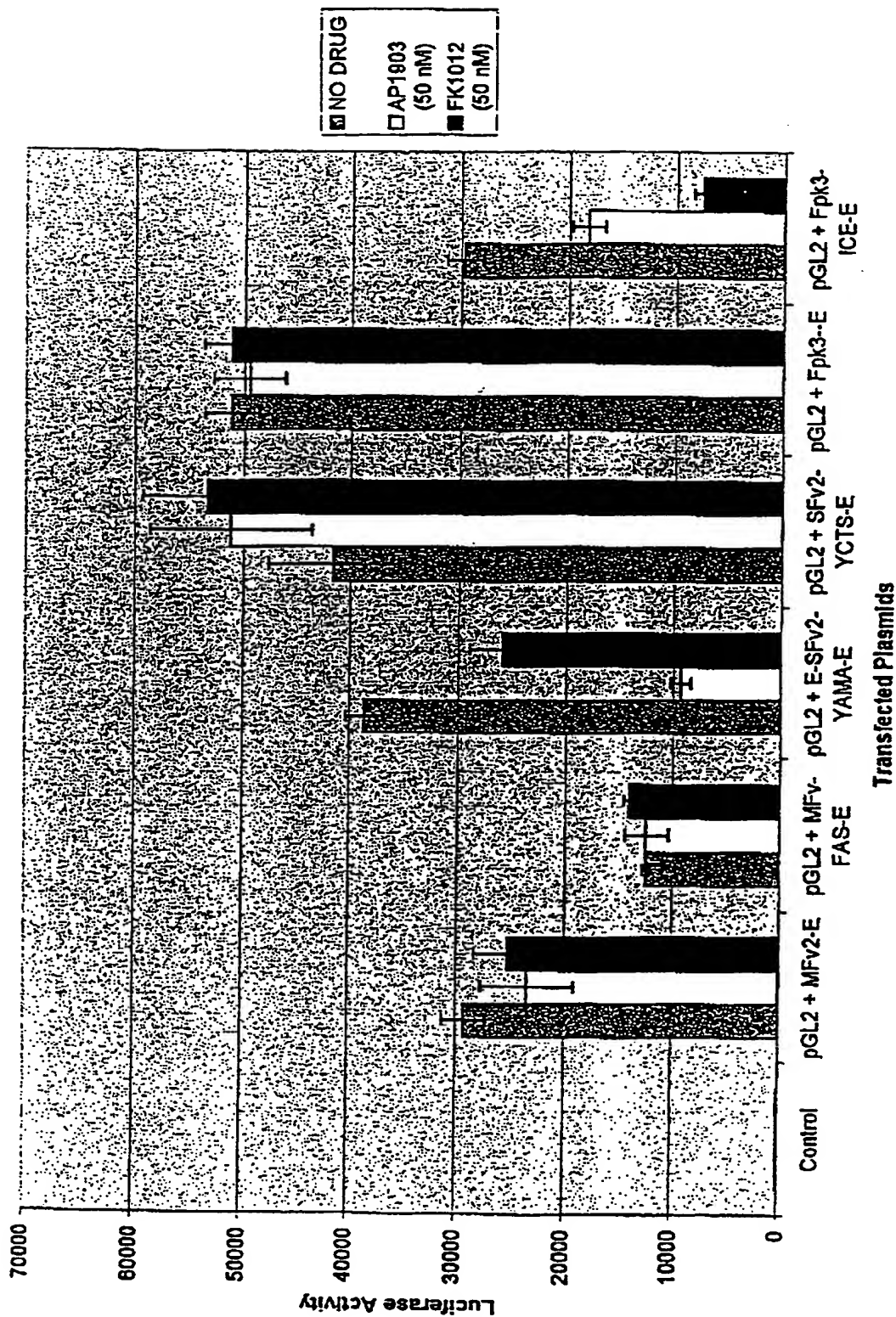


FIGURE 21

CID-Mediated Apoptosis In BPH-derived CR-2a SMC



006260" BT44360

FIGURE 22

CID-Mediated Apoptosis in BPH derived JD SMC

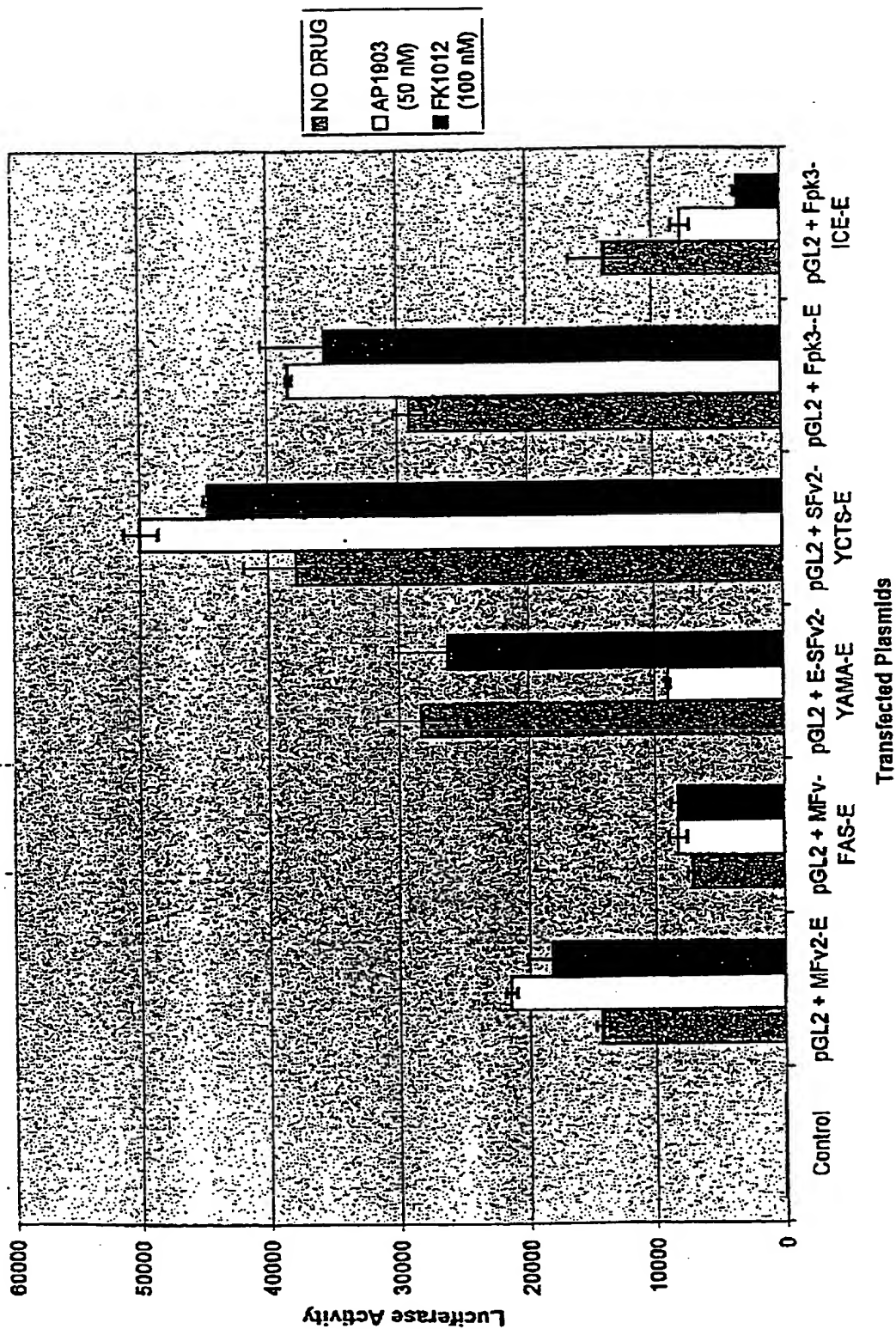


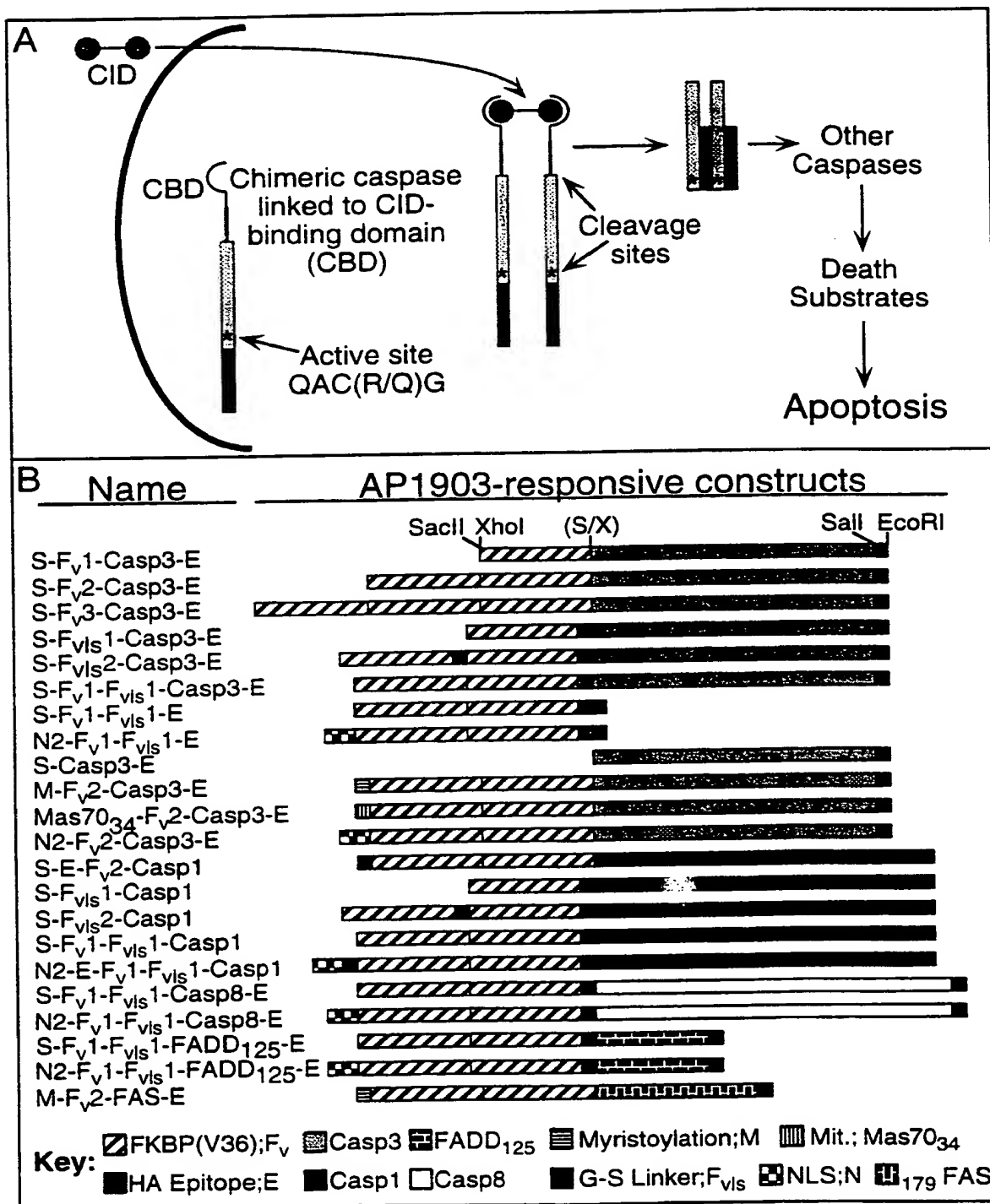
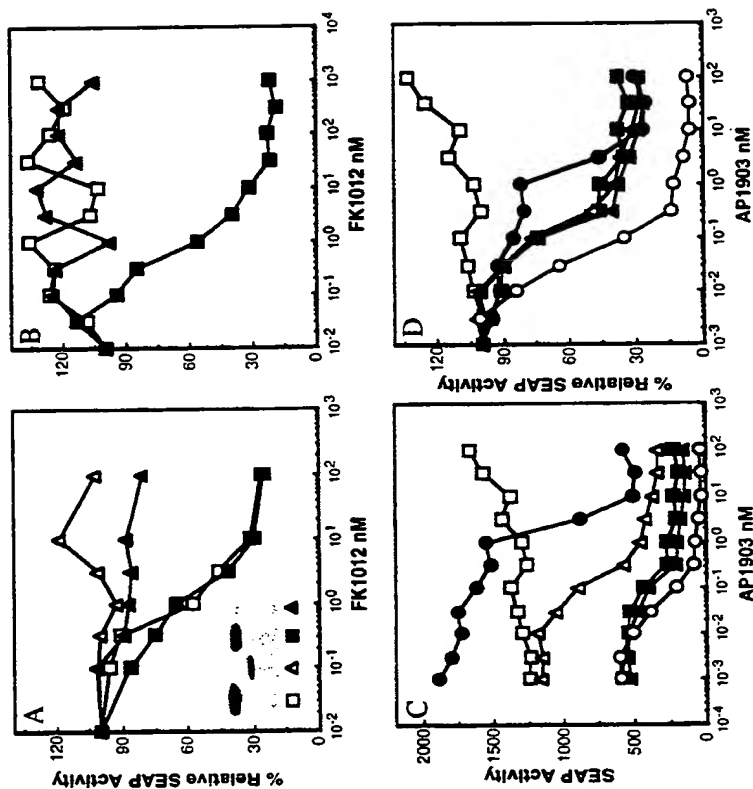
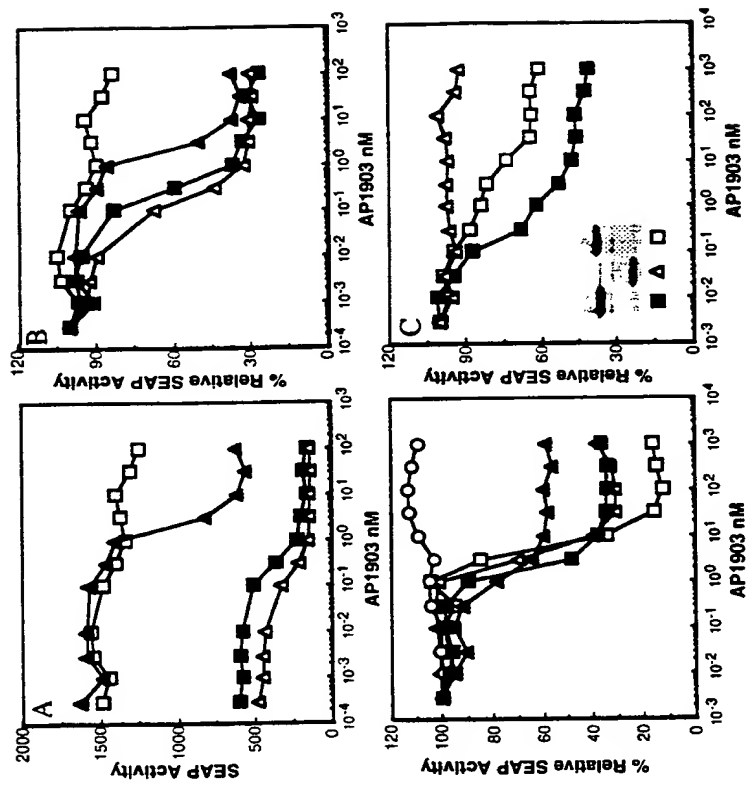
FIG 23
(FIG 24 on Reverse)

Fig 25 (Fig 26 on Reverse)



Final Size: 67%
or 7 cm

Fig 27



Final Size: 67%
or 6.9 cm

Generation of Ad-GFP-CMV-YM-E

Ad-GFP-CMV-E-ICE US 99/06799

pAdTrack-CMV ~ Fv1s1-YM-E

~ E-Fv1-Fv1s1

-ICEst

① cut pAdTrack-CMV with EcoRV + Not I

1 μ g/2 μ l (pAdTrack-CMV) + 1 μ l Buffer H
 + 1 μ l EcoRV + 1 μ l Not I + H₂O 5 μ l
 37°C, 2 hr



pAdTrack-CMV
 9220 bp

② cut pSH1/s-Fv1s1-YM-E with Not I + EcoRI

(got 2.2 kb Fv1s1-YM-E)

(E-Fv1-Fv1s1-ICEst)

or

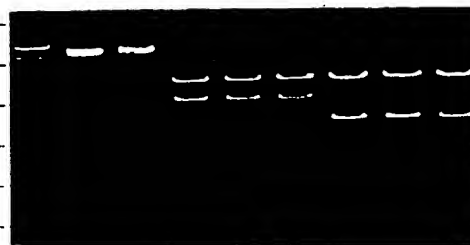
2 μ g/12 μ l (pSH1/s-Fv1s1-YM-E) + 2 μ l EcoRI
 + 2 μ l Buffer H + H₂O 14.8 μ l, 37°C, 1 hr

2 μ g/3.2 μ l
 12.8 H₂O

Blunt

+ 10 μ M dNTP (2 μ l (10 μ M/each) + 2 μ l Buffer B14 μ l H₂O + 4 U/2 μ l Klenow, 25°C, 30 min; + EDTA \rightarrow 10 minInactivate Klenow by heating at 75°C, 10 min. Phenol:Chloroform extraction
 (see Gene Clean)Then + 2 μ l Not I, 37°C, 1 hr

③ Run gel, cut bands, pool together,
 Gene Clean. Elute in 40 μ l



pSH1/s-Fv1s1-YM-E
 + Not I + EcoRI

④ Ligation

Eluent 16 μ l + 2 μ l 10X Ligation Buffer
 + 2 μ l Taq ligase, 16°C, overnight

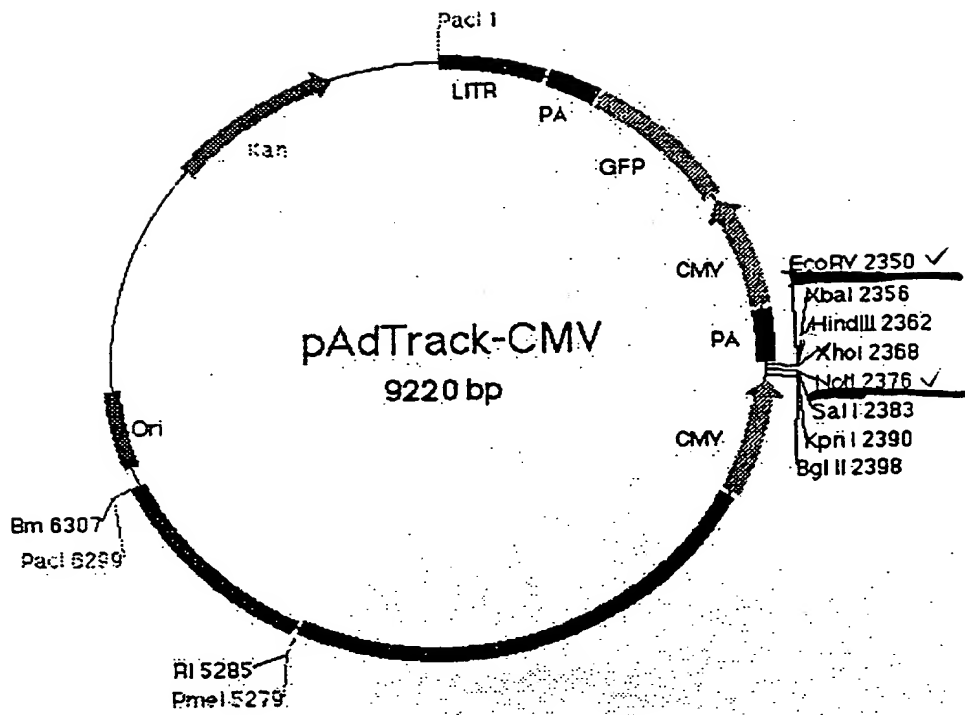
pAdTrack-CMV
 + Not I + EcoRV
 ↓
 pSH1/s-E-
 Fv1-Fv1s1-
 -ICEst
 + Not I
 + Sac I

pSH1/s-
 Fv1s1-
 -YM-E
 + Not I
 + Sac I

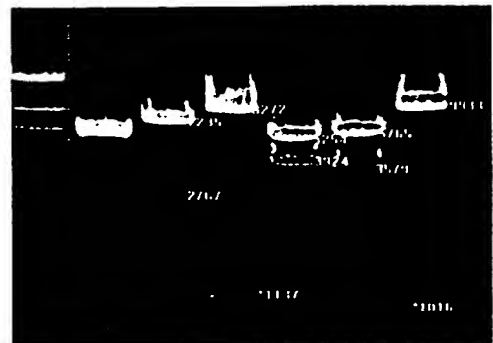
⑤ Transformation

⑥ Mini-prep with Qagen kit

FIG 28



pAdTrack-CMV-SFV/Sl-YM-E



F. G - 29

JPEG image 511x431 pixels

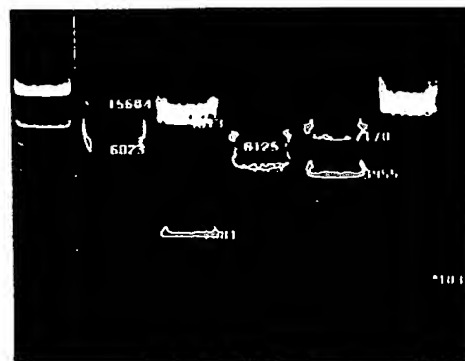
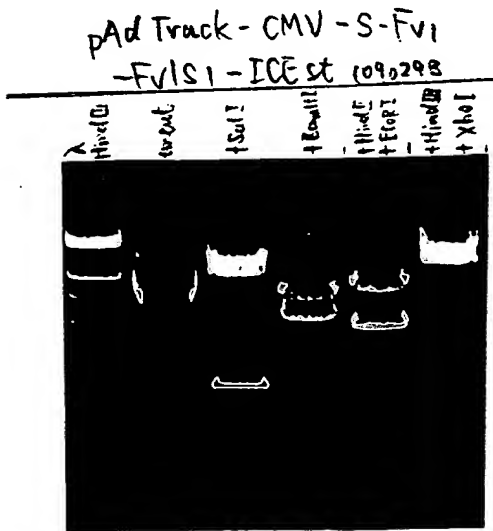
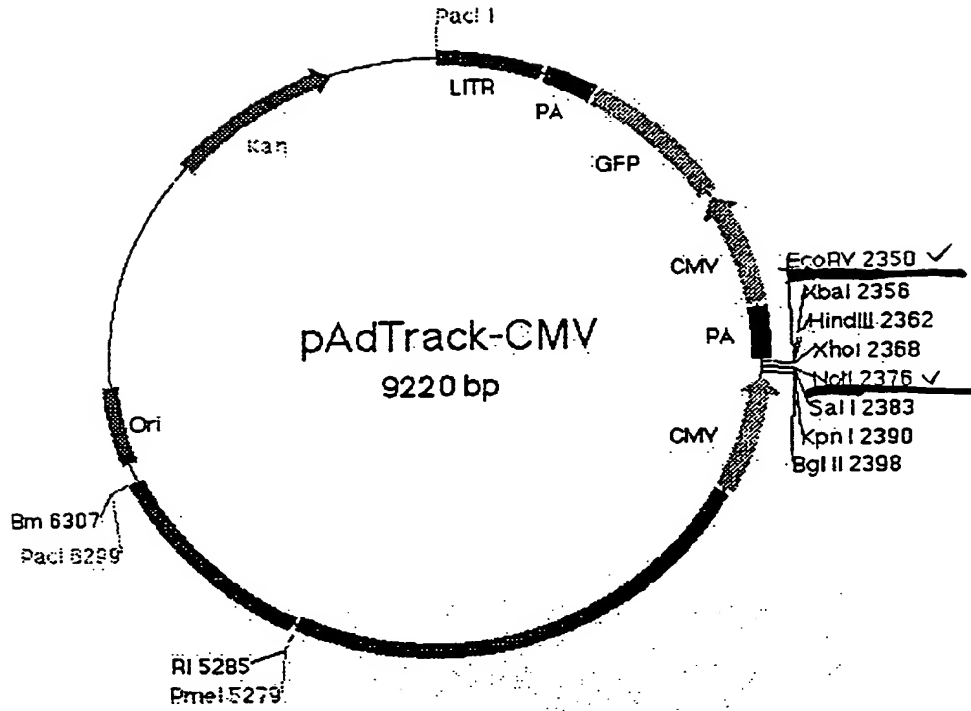


FIG 30

A 3

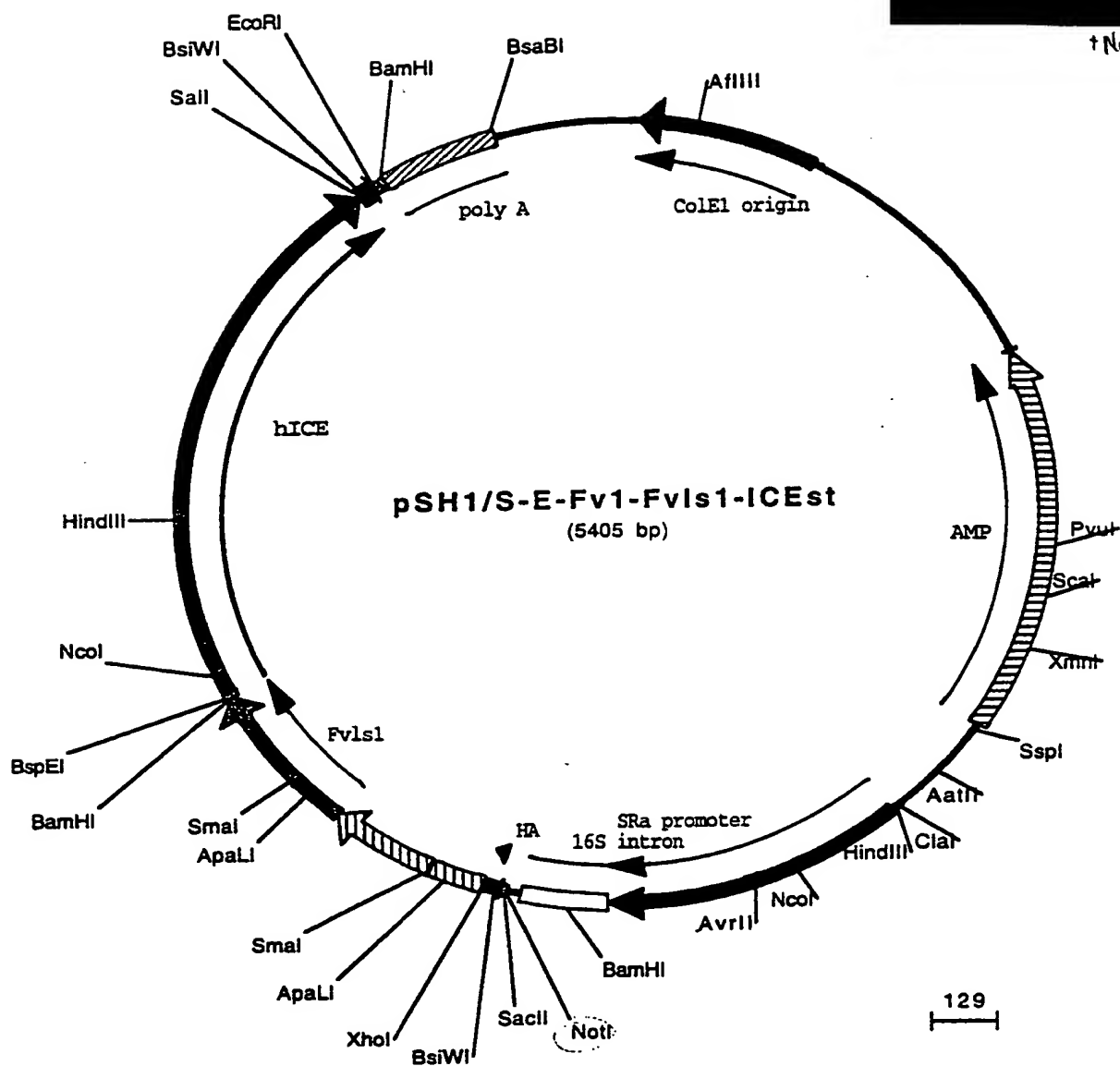
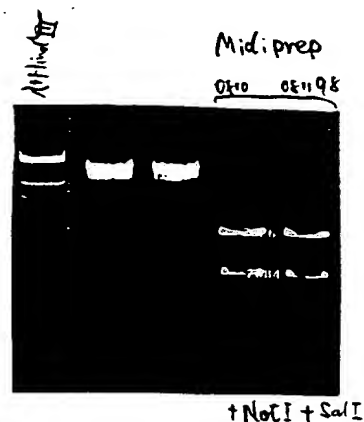


FIG 31

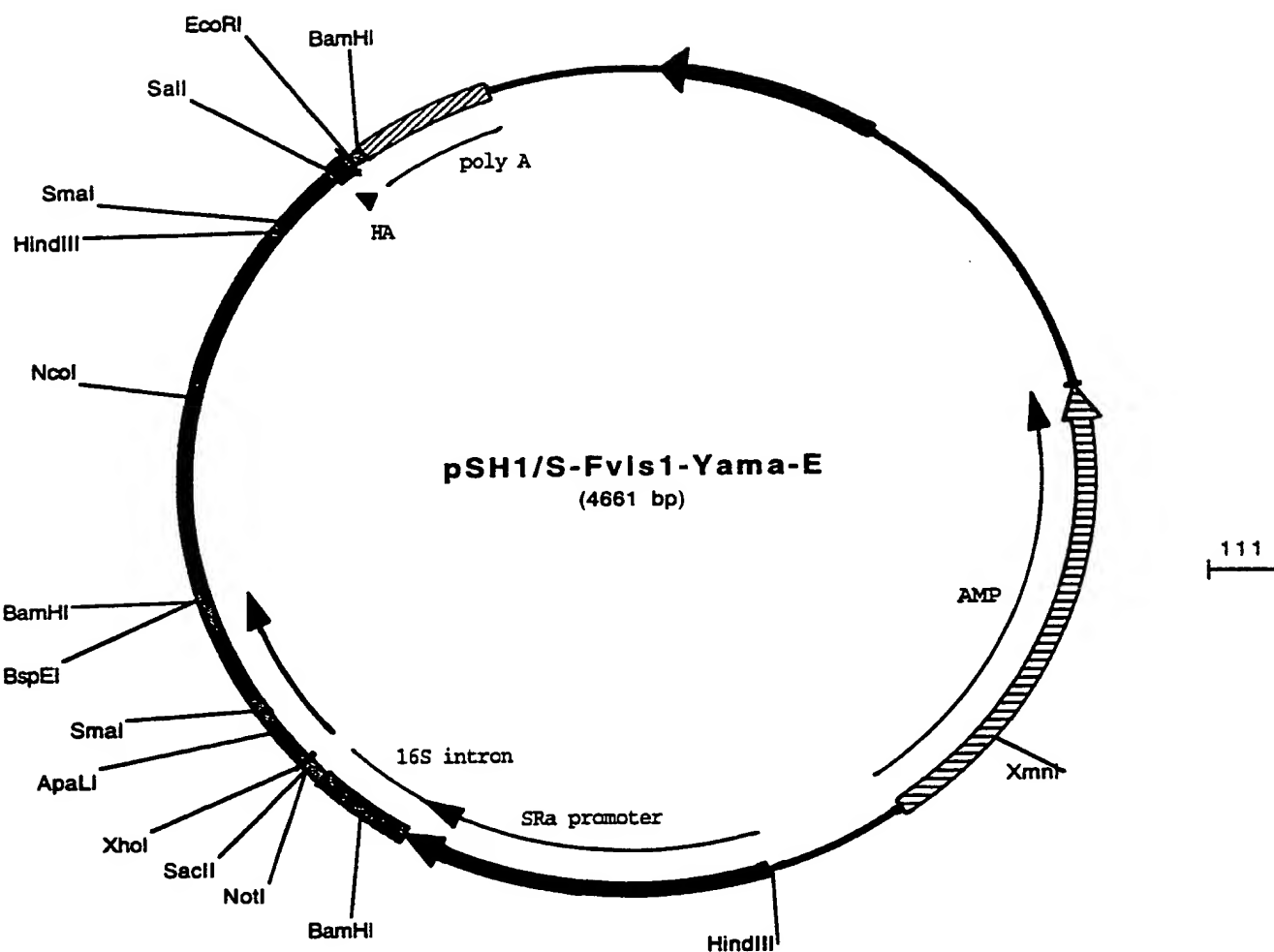


FIG 32

00647418-09200

Generation of Ad-GFP-CMV-YM-E Ad-GFP-CMV-E-ICE

① Co-transform E. coli BJ5183 with pAdEasy-1 and pAdTrack-CMV-Fv1s1-YM-E or pAdTrack-CMV-E-Fv1-Fv1s1-ICE.

a. Linearize the shuttle plasmids with Pme I

1 μ g of pAdTrack-CMV-Fv1s1-YM-E

or pAdTrack-CMV-E-Fv1-Fv1s1-ICE

+ 1 μ l Buffer + H₂O \rightarrow 9 μ l, + 1 μ l Pme I

37°C, 2 hr.

phenol-chloroform extraction, ethanol precipitation and resuspend in 6 μ l H₂O.

b. Co-transformation: with 100 ng pAdEasy-1

20 μ l of BJ5183

2.0 mm cuvettes

at 250V, 200 Ohms, 25 μ FD

c. Selection:

pick 10 smallest colonies, miniprep, check with Pac I.

re-transform XL1-Blue with the correct plasmid, miniprep, recheck with Pac I.

Midiprep.

d. Transfect 293 cells with FuGene.

90% confluence 293 cells in 6-well-plate,

4 μ g plasmid/6 μ l FuGene / well.

FIG 33

U9/647418

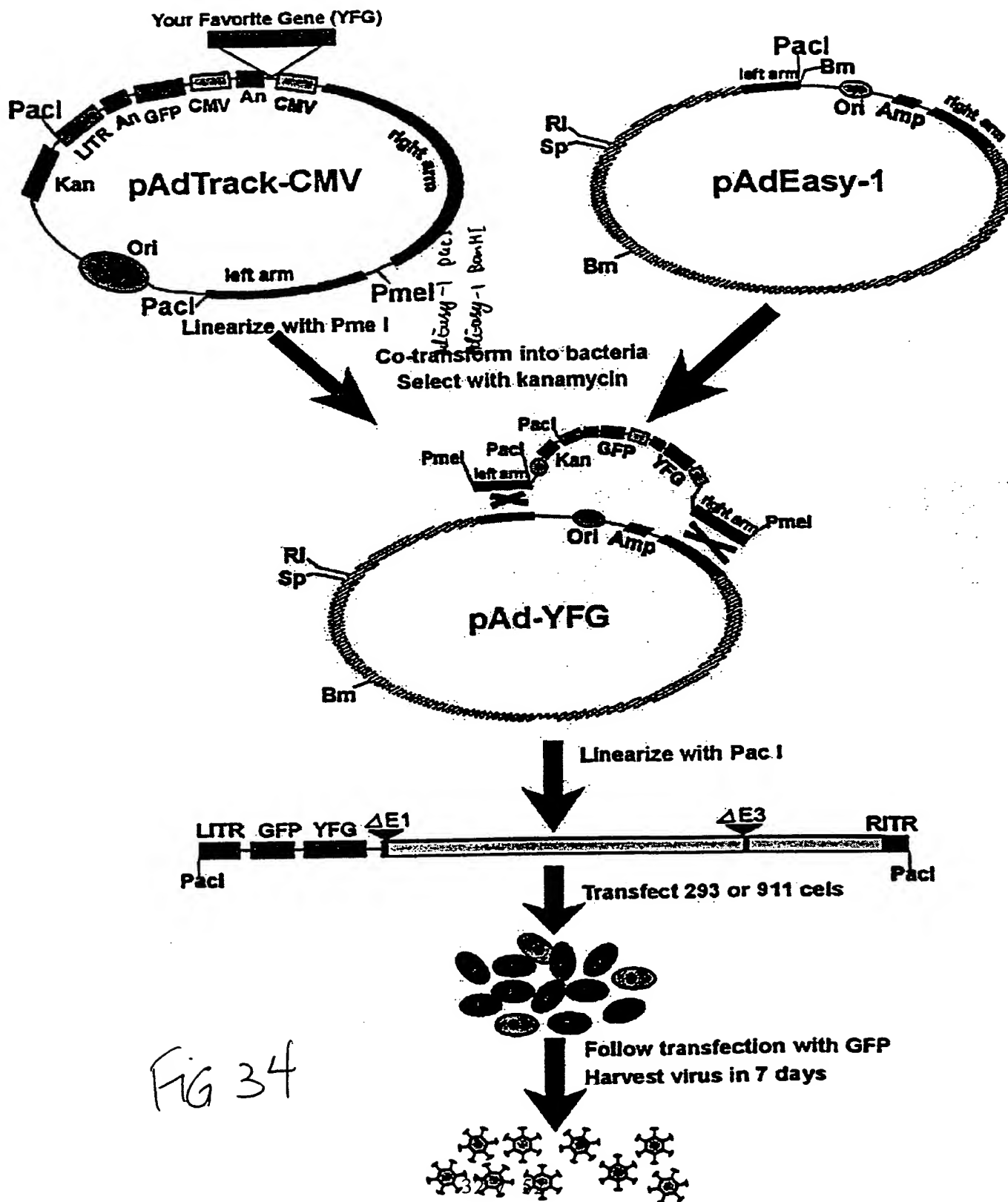


FIG 34

006260" 87424960

+



Midi prep
082498

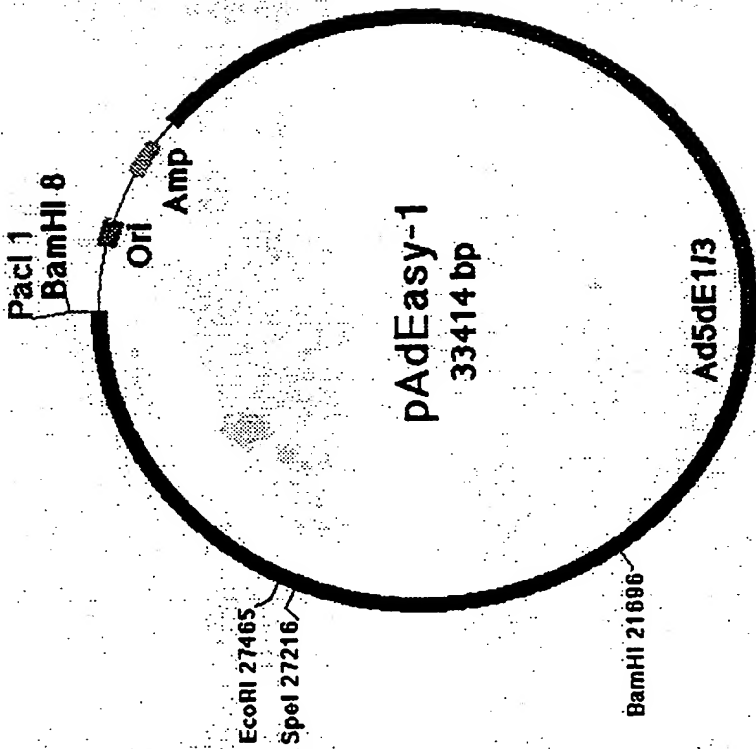


Fig35

pAd Easy-1-Track-CMV-ICE-E
-YAMA-E

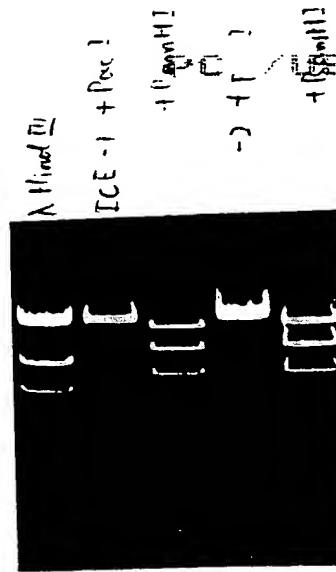
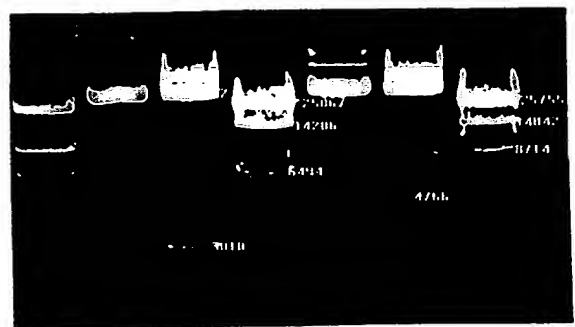
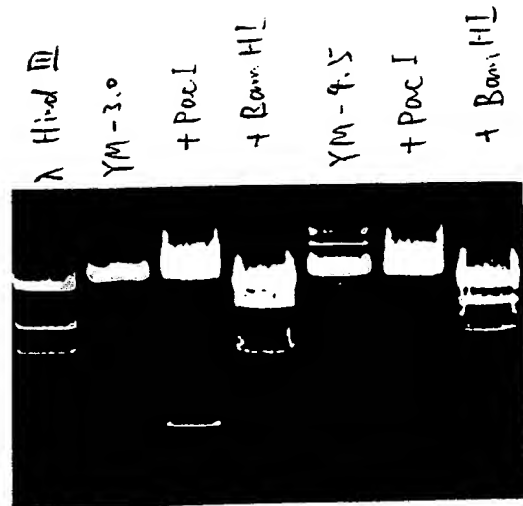


FIG 3b



006260" 87424950

FIG 37 Generation of Ad-CMV-E-Fv1-Fv1s1-ICEst

Construction of pshuttle-CMV-E-Fv1-Fv1s1-ICEst and Ad-CMV-E-ICE

① cut pshuttle-CMV with EcoRV + Not I

1 μ g / 2 μ l (pshuttle-CMV) + 1 μ l Buffer H
+ 1 μ l EcoRV + 1 μ l Not I + H₂O 5 μ l
37°C, 2 hr

② cut pSH/S-E-Fv1-Fv1s1-ICEst with Not I + EcoRV (get E-Fv1-Fv1s1-ICEst)

3 μ g / 4 μ l + 3 μ l Buffer H + 3 μ l EcoRV
+ H₂O 21 μ l, 37°C, 1 hr

Blunt

+ 10 mM DTT 15 μ l (10 mM / each) + 3 μ l Buffer 2
+ 3 μ l H₂O + 7 μ l T4 DNA Polymerase, 25°C, 30'
+ EDTA \rightarrow 10 mM, 75°C, 10'
phenol:chloroform extraction (twice)
ethanol precipitation \rightarrow 24 μ l H₂O

Then

+ 3 μ l Buffer H + 3 μ l Not I
37°C, 2 hr

③ Run Gel, cut bands, pool together, GeneClean II into 4 μ l H₂O

2) Ligation

Elution: 16 μ l + 2 μ l 10x Ligation Buffer
+ 2 μ l T4 Ligase, 25°C overnight

④ Transformation

E. Miniprep + Restriction Enzyme checking

⑦ cut pshuttle-CMV-E-Fv1-Fv1s1-ICEst with PmeI (1/2 of a miniprep), Cotransform E. coli BJ5183 with
100 ng pAdEasy-1. Miniprep + PacI checking (photo above)

⑧ chose #2, retransform XL-1 Blue, Miniprep, + PacI; Midi prep, + PacI checking (small photo above)

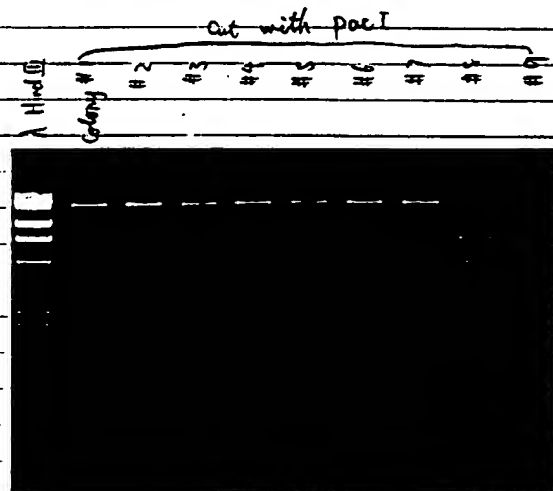
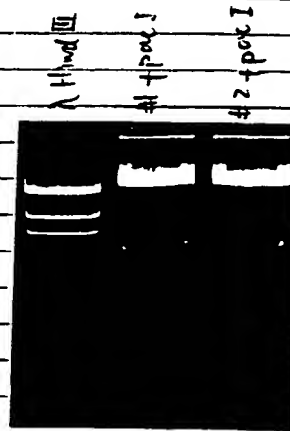
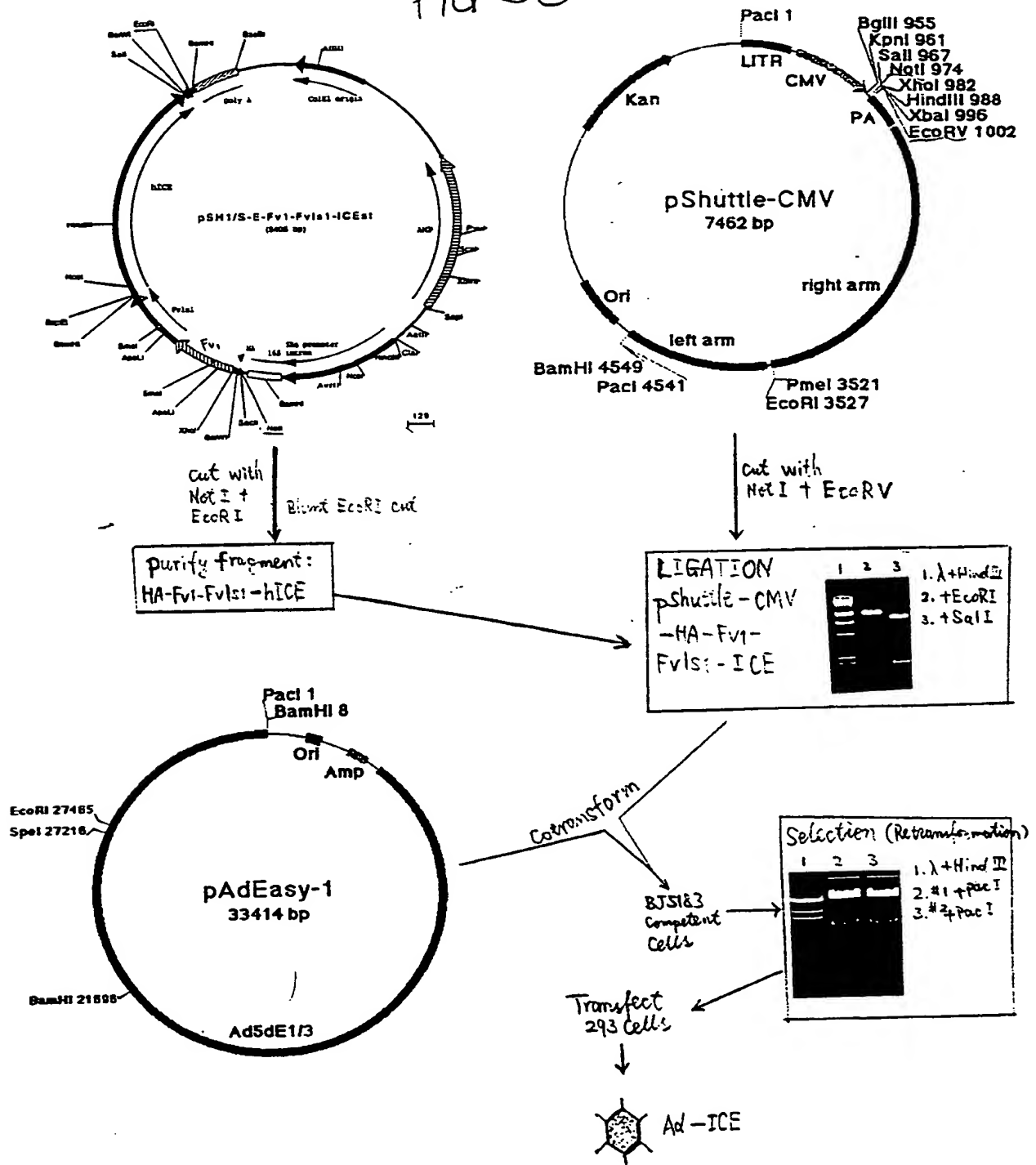


Fig 38



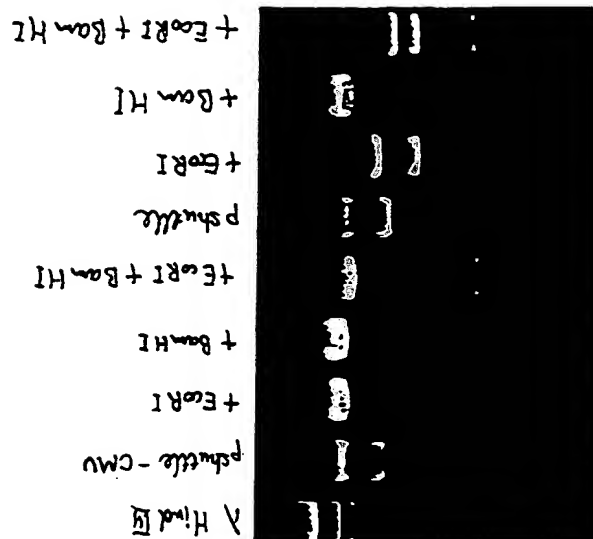
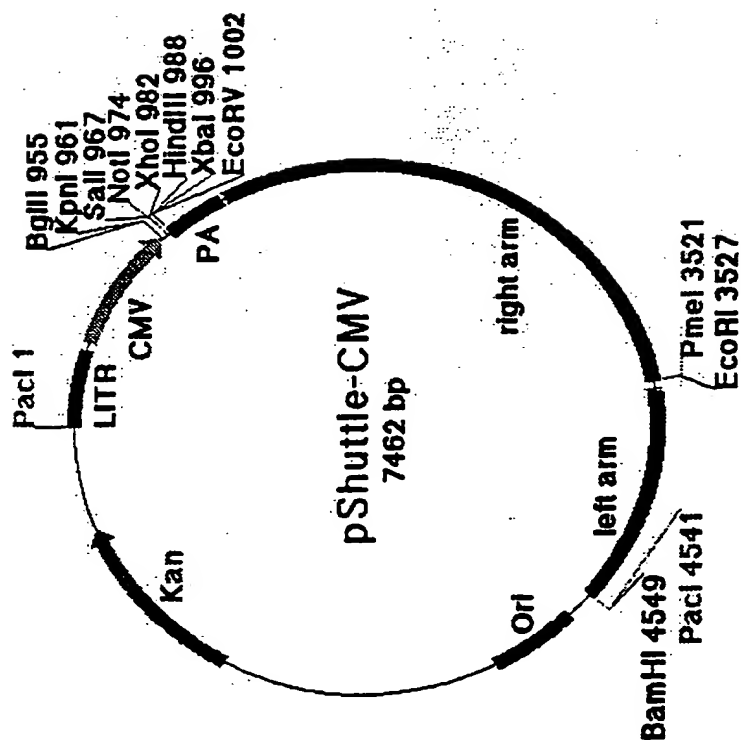


Fig 39

FIG 40

Luciferase Assay of pSiville-CMV

- FV₁ - FVSI - ICE - E

0104 - 010799

- ① 1×10^5 JD-201 cells / well (6 well - plate)
in 1640 RPMI media with 5% FCS
incubate 24 hrs

- ② Transfection: 2 μ g pGL₂ 2 μ g ICE per well
Fugene 2ul / μ g DNA

Incubate 6 hrs, add AP1903 at 50 nM.

Incubate 24 hrs.

- ③ Lysis - Autometer

8 JAN 99 AM 10:59

V. 1.1

MEAS. TIME(s) : 15.0

SAMPLE RLU

% C.V.

1 1 294

1 2 291

MEAN 292

0.8

Control

2 1 2681959

2 2 2769999

MEAN 2725979

2.3 Control + pGL₂

3 1 208762

3 2 223711

MEAN 216237

4.9 pGL₂ + ICE

4 1 22084

4 2 22859

MEAN 22471

2.4 pGL₂ + ICE + AP1903

006260" 8TH4960

FIG 41

JD-2A, T-C₂, T-C₂G, LNCaP — Adeno-TM or Adeno-ICE

① Plating cells: 24 well-plate

JD-2A — 20000/well

T-C₂ > 10000/wellT-C₂G

LNCaP — 30000/well

in 1ml RPMI 1640 Media (5% FBS)

in 1ml DMEM Media (+5% FBS, 5% Nucserum, Insulin
DMF)

incubate JD 24 hrs

T-C₂, T-C₂G — 48 hrs

LNCaP — 72 hrs

Cell number doubling — 40000

— 40000

— 60000

② Infection:

For JD: ICE 1×10^7 iu/ml

MOI

1

2

4

8

16

Add

4

8

16

32

64 μ lYM 2×10^7 iu/ml

MOI

2.5

5

10

20

40

5

10

20

40

80 μ l

1:10

For T-C₂ ICE 5×10^7 iu/ml

MOI

2.5

5

10

20

40

+ T-C₂G

Add

2

4

8

16

32

YM 5×10^7 iu/ml

MOI

2.5

5

10

20

40

Add

2

4

8

16

32

For LNCaP

3

6

12

24

48

After 4 hrs. Add AP 1903 \rightarrow 50 nM, incubate 24 hrs.

③ Fix with 1% Glutaraldehyde — PBS, 15';

Stain 0.5% Crystal violet, 20';

Wash with H₂O, 30';Resolve with Safran's Solution, 200 μ l/well, 5';Transfer 100 μ l to each well in 96-well plate. Read OD at 570 nm.

006260" 2424960

T-C2G Cells Treated with Ad-YAMA or Ad-ICE + AP1903 or AP20187

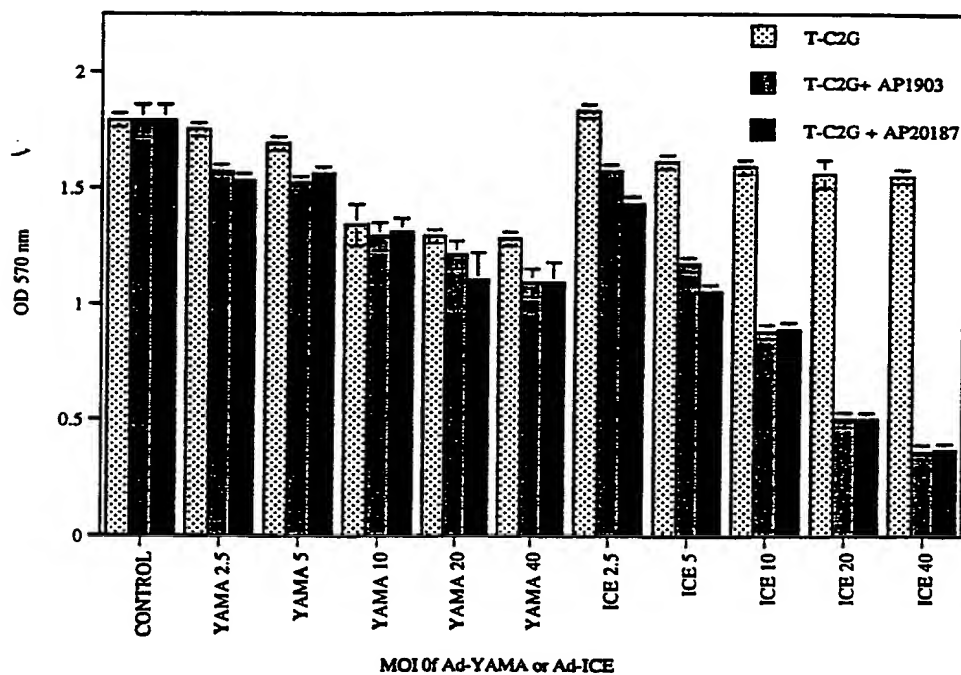


Fig 42

09/647418

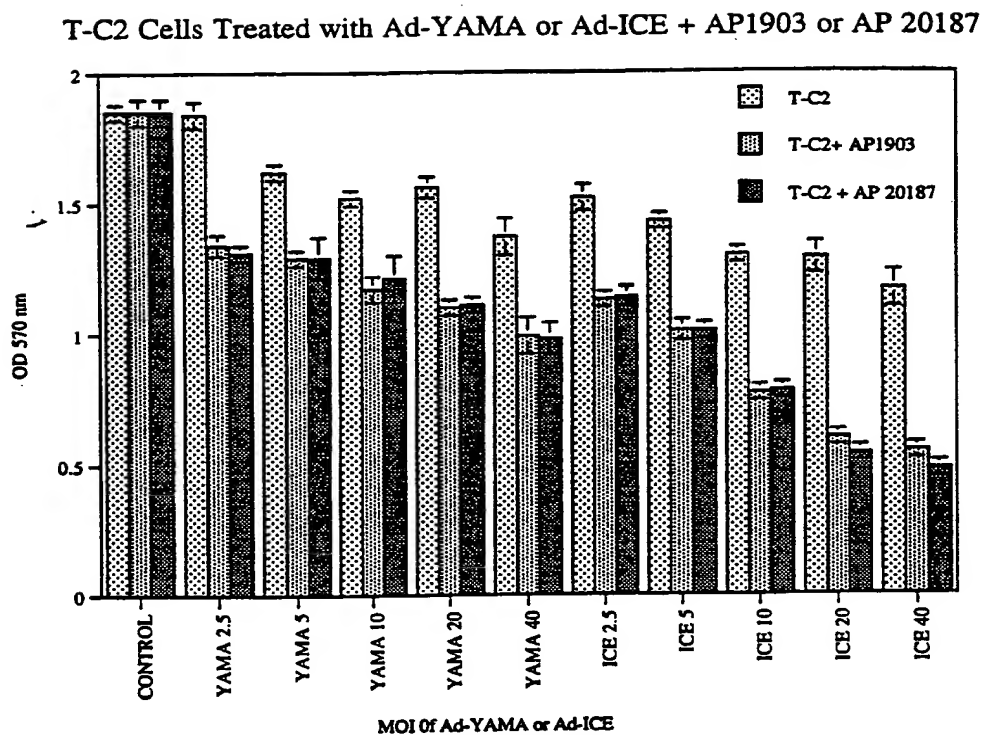


Fig 43

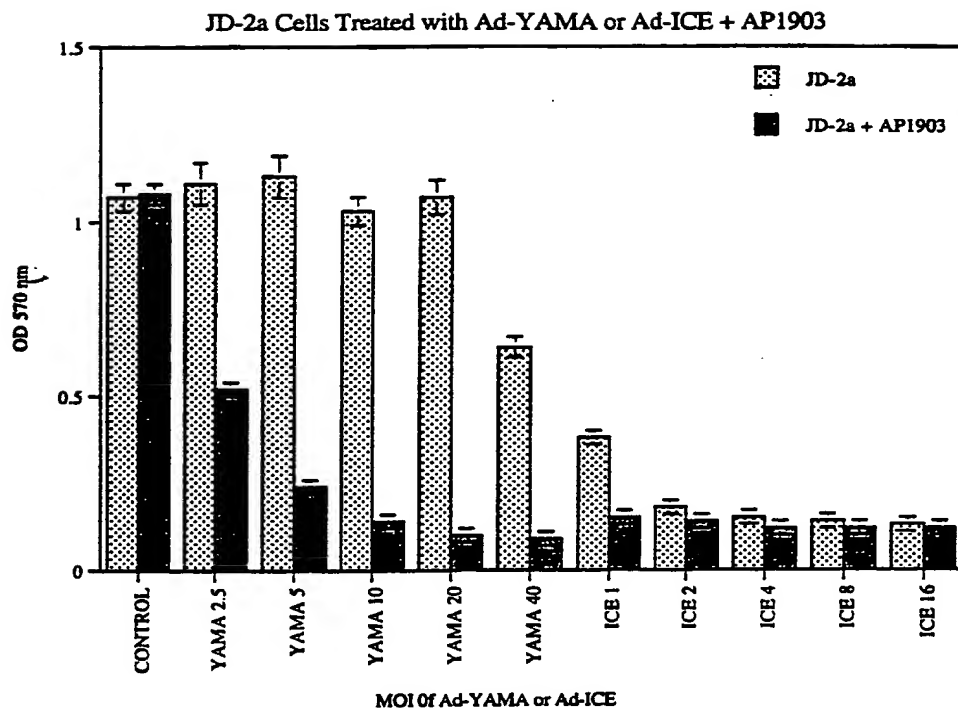


FIG 44

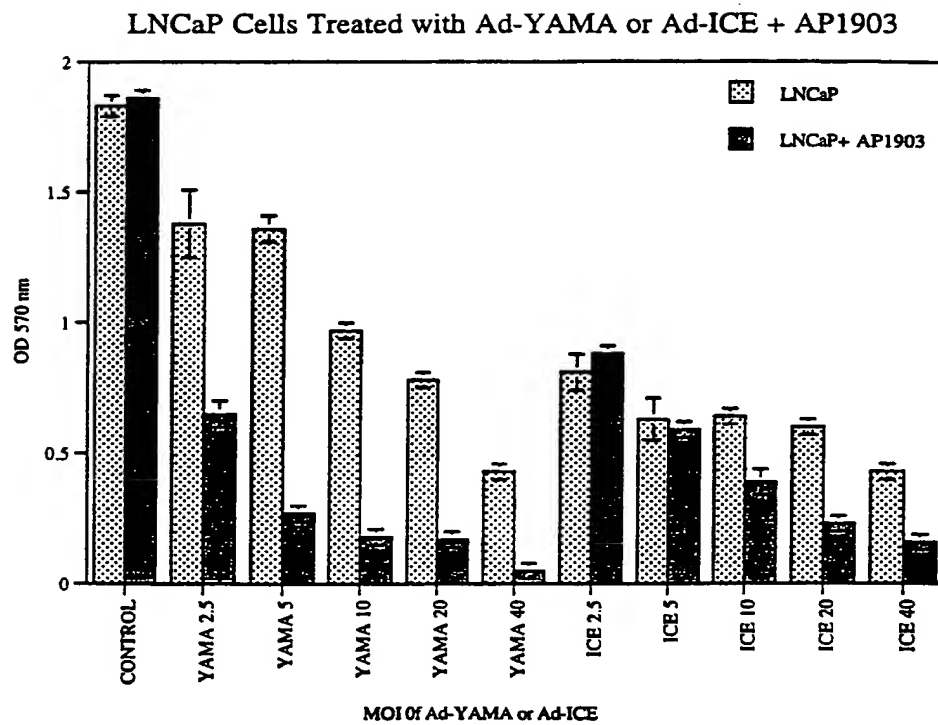


Fig 45

09/647418

WO 99/50425

PCT/US99/06799

44 / 50

Western Blot

1103 - 110495

293 cells infected with Ad-YM or Ad-ICE

9% Resolving Gel; Transfer - 400 mA, 2 hrs.

1:1000 α -HA11 - 1st Ab.

1 Ad-YM3

2 - +1903 (100 nM)

3 - +2-D-DCB (100 nM)

4 - +Both

5 Ad-ICE

6 - +1903

7 - +2-D-DCB

8 - +Both

9 Ad-YM4

10 - +1903

11 - +2-D-DCB

12 - +Both

Expression and Activation of ICE and YM

KODAK SAFETY FILM

KODAK SAFETY FILM

FIG 4b

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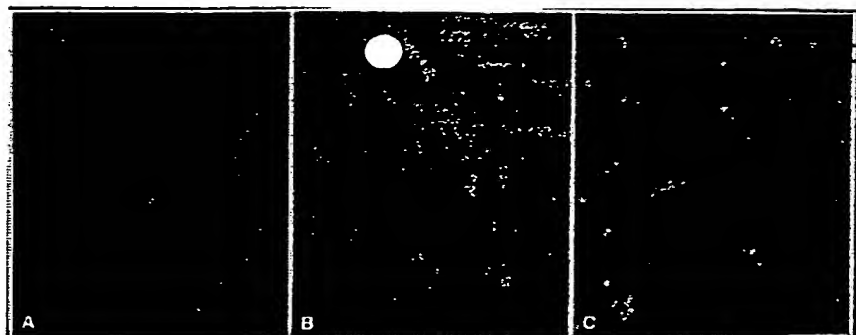


FIG 47

006260" BT-4960

ADV-FKBP/ICE effectively kills PC-3 prostate cancer cells

MOI:
AP1903
(50 nM)



Fig 48

ADV-FKBP/ICE effectively kills JD-2a BPH cells

MOI:
AP1903
(50 nM)

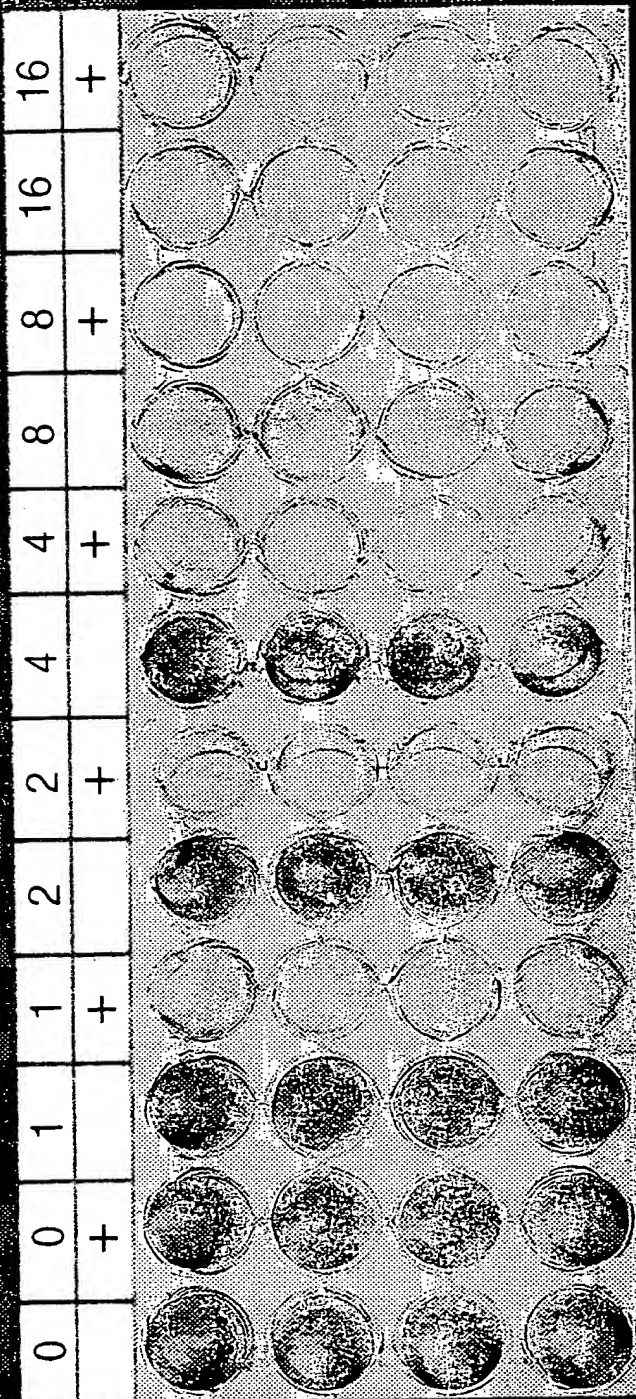
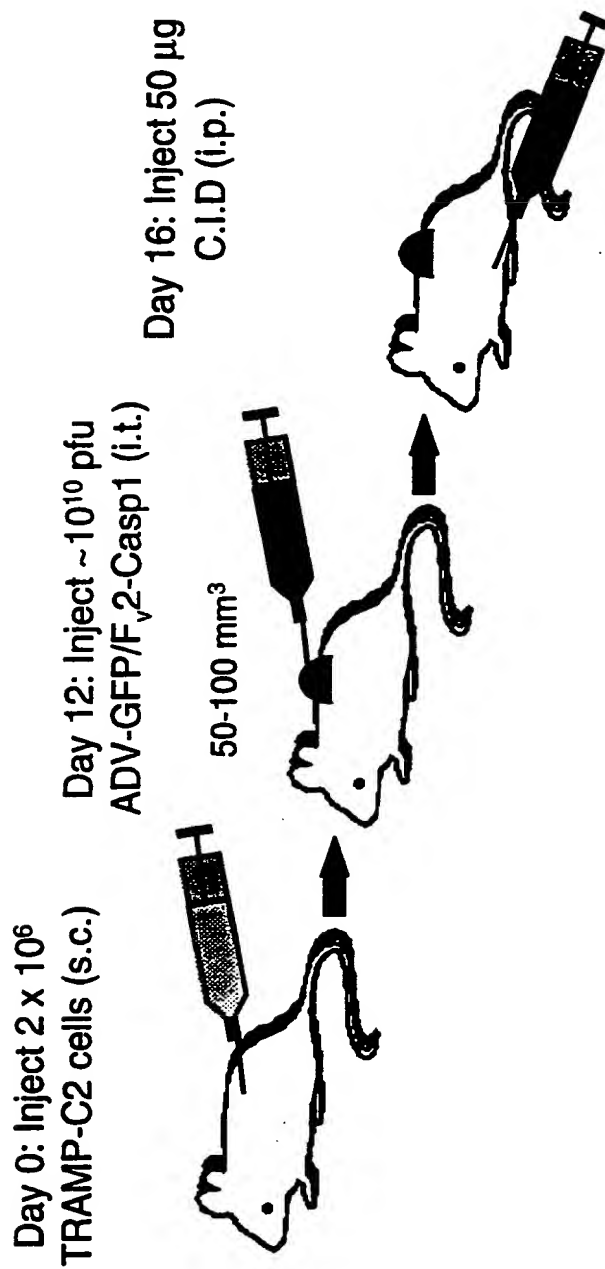


FIG 49

Treatment of s.c. prostate adenocarcinoma in situ with CID-inducible caspases



Analysis: resect tumor, H&E, TUNEL, anti-GFP

Fig 50

ADV-FKBP/ICE effectively kills TRAMP-C2 cells in vivo

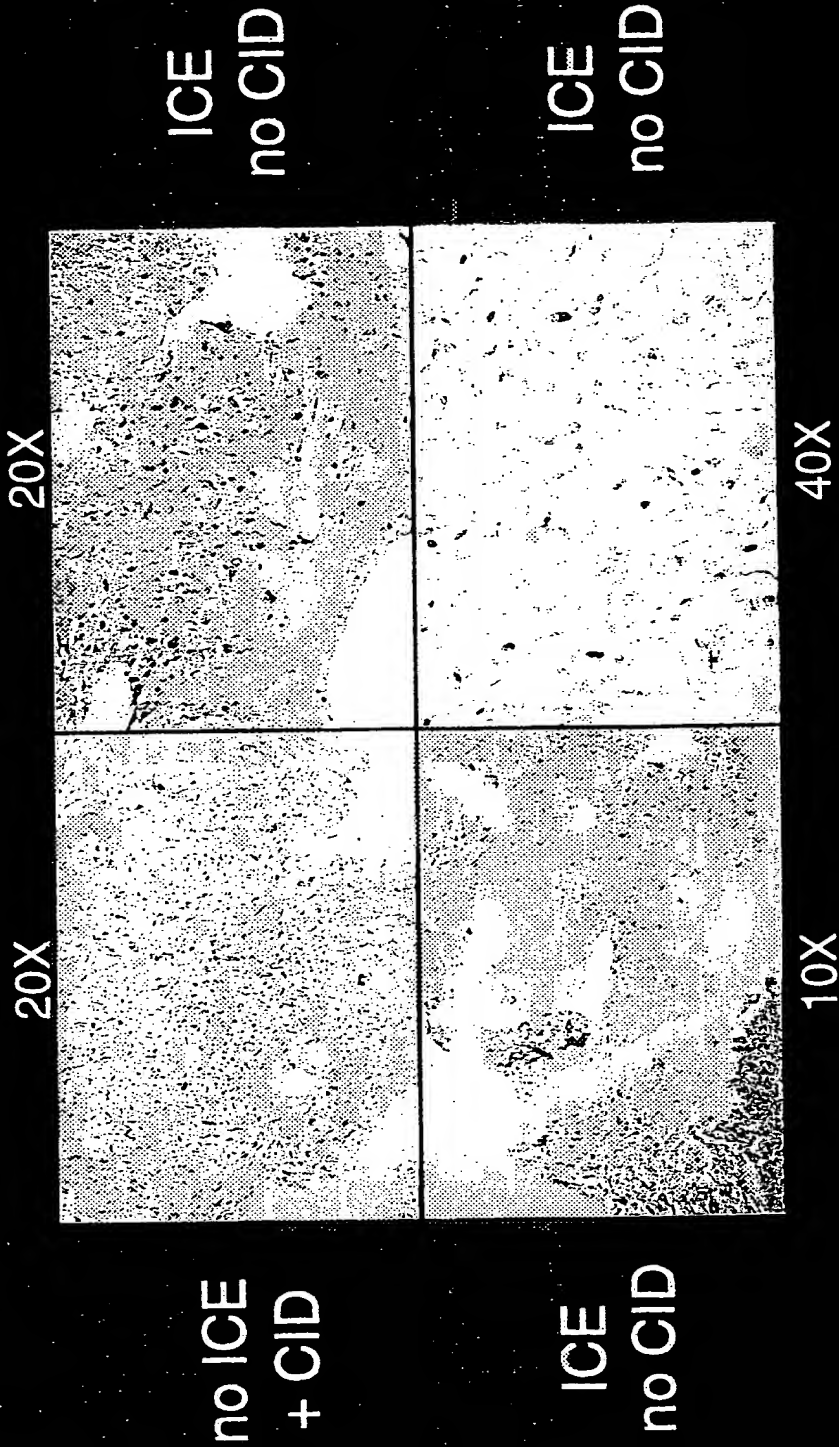


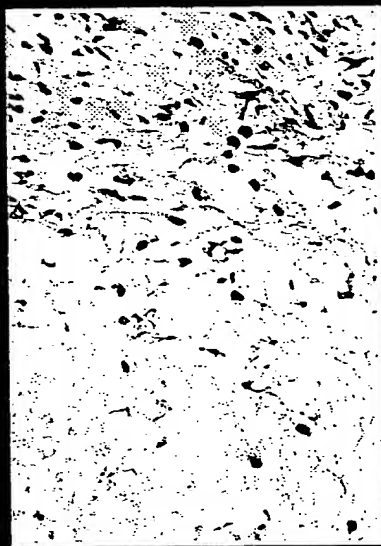
Fig 51

*ADV-FKBP/ICE + CID very effectively
kills TRAMP-C2 cells in vivo*

20X

ICE
+ CID

40X

ICE
+ CID

20X

ICE
+ CID

40X

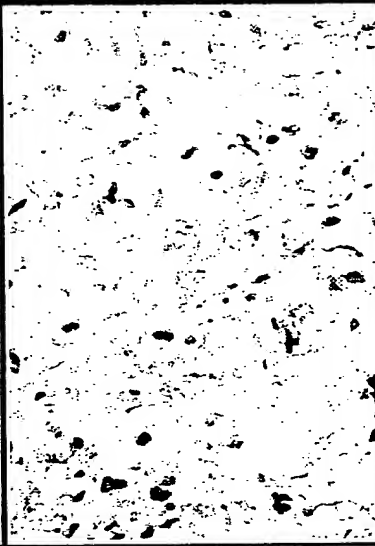
ICE
+ CID

Fig 52

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